EFFECT OF EXOGENOUS L-ORNITHINE L-ASPARTATE ON ETHANOL INDUCED TESTICULAR INJURY IN WISTAR RATS

Maneesh Mailankot, H Jayalekshmi*, Amit Chakrabarti** and D M Vasudevan***

Department of Biochemistry, Melaka Manipal Medical College, Manipal-576 104, *Amrita School of Biotechnology, Amritapuri-690 525, **Department of Pharmacology, Sikkim Manipal Institute of Medical Sciences, Gangtok-737 102, ***Department of Biochemistry, Amrita Institute of Medical Sciences, Cochin- 662 026,

ABSTRACT

To investigate reversibility of ethanol induced testicular injuries on treatment with L-ornithine-L-aspartate, male Wistar rats were treated with ethanol (1.6g/kg b.wt/day) and L-ornithine- L-aspartate (200mg/kg b.wt/day) for 4 weeks. L-ornithine-L-aspartate effectively prevented the ethanol induced body and testes weight reduction; changes in testicular weight well correlated with body weight. Drug exhibited an ability to counteract ethanol induced oxidative challenge as it effectively reduced testicular TBARS and increased tissue ascorbic acid, GSH and activities of superoxide dismutase, catalase, GSH-Red and Se-GSH-Px. However the drug didn't show promising effect on inhibitory effect of ethanol on testicular D⁵, 3-beta and 17-beta HSD (hydroxy steroid dehydrogenase).

KEY WORDS

Ethanol, Oxidative damage, Testicular injury, L-ornithine-L-aspartate treatment.

INTRODUCTION

Alcohol abuse is well known to impair reproductive function (1) and alcoholics are found having fertility abnormalities with low sperm count and impaired sperm motility(2). In our previous study(3) with chronic ethanol treated rats we reported reduction in body and testis weight, decrease in testicular ascorbic acid, reduced glutathione (GSH) and activities of superoxide dismutase (SOD), catalase, glutathione reductase (GR), glutathione peroxidase (GPx), 3 β -hydroxy steroid dehydrogenase (3 beta-HSD) and 17 β -hydroxy steroid dehydrogenase (17 beta-HSD).

L-ornithine-L-aspartate is stable salt of the amino acids ornithine and aspartic acid and is a nutritional supplement that has been investigated as a treatment for cirrhosis and hepatic encephalopathy (4). Oral L-ornithine-L-aspartate is a

safe, well-tolerated treatment with a good compliance rate and a beneficial therapeutic effect in patients with cirrhosis(4). The liver is exposed to the higher concentrations of alcohol in blood and is responsible for more than 95% of its metabolism (5). 75% of the men with advanced alcoholic cirrhosis have been reported to have testicular atrophy (6). In view of the possible role of liver in ethanol induced testicular injury the present study was designed to investigate the effect of L-ornithine-L-aspartate on ethanol induced testicular injury in Wistar rats.

MATERIALS AND METHODS

Fine chemicals were purchased from Sisco Research Laboratory, India and Sigma Chemical Co., St. Louis, USA. All other chemicals used were of analytical grade and were purchased from Merck Ltd., India and Sisco Research Laboratories Ltd., India. L-ornithine-L-aspartate (150mg/tab) was procured from HepaMerz, Win-Medicare, India.

Male Wistar rats (10-12 weeks of age) weighing 100-120 g were used. The animals were housed in plastic cages of size 14"x9"x8" (6 rats in each cage) in side a well-ventilated room. The room temperature was maintained at 22 $\pm$ 2°C with a 12-12 hr L: D cycle. All rats had free access to a standard diet

Address for Correspondence :

Dr. Maneesh Mailankot
Department of Biochemistry,
Melaka Manipal Medical College,
Manipal-576 104
E-mail: manu_only@hotmail.com

and water. National Institutes of Health (NIH), Bethesda, MD, USA guidelines were followed for maintenance, handling, experimentation, sacrifice and disposal of animals. The experimental study protocol was approved by the Institutional Animal Ethics Committee, SMIMS, Gangtok.

The dose of ethanol was determined as described previously (4). The animals were divided into 5 groups of 6 each i.e. Group I (Control): 1g double distilled water/kg b.wt/day for 4 weeks, orally; Group II: Ethanol treated rats (1.6g ethanol/kg b.wt/day for 4 weeks, orally); Group III: Ethanol + L-ornithine-L-aspartate treated rats (1.6g ethanol + 200mg L-ornithine-L-aspartate/kg b.wt/day for 4 weeks, orally); Group IV: Ethanol followed by L-ornithine-L-aspartate treated rats (1.6g ethanol/kg b.wt/day for 4 weeks followed by 200mg L-ornithine-L-aspartate/kg b.wt/day for next 4 weeks, orally); Group V: Ethanol treatment (1.6g ethanol/kg b.wt/day, orally) for 4 weeks and followed by 4 weeks abstinence.

Rats were weighed and killed by cervical dislocation under light ether anesthesia; kidney were removed, cleaned of the adhering tissues and weighed. Tissues were immediately rinsed with ice cold normal saline, trimmed and processed for biochemical analysis.

Biochemical analysis- Total protein (7), extent of lipid peroxidation (8), ascorbic acid (9), reduced glutathione (GSH) (10), catalase (EC 1.11.1.6) (11), superoxide dismutase (SOD, EC 1.15.1.1) (12), glutathione peroxidase (Se-GSH-Px, EC 1.11.1.9) (13), glutathione reductase (GSH-Red, EC 1.6.4.1) (14), glutathione-S-transferase (GSH-ST, EC 2.5.1.18) (15), D5, 3 β -hydroxy steroid dehydrogenase (D5, 3 β -HSD) (16) and 17 β -hydroxy steroid dehydrogenase (17 β -HSD) (17) were estimated.

The data were expressed as mean $\pm$ SD. Statistical analysis was performed using Student's 't' test for unpaired data. Significance of difference was set at $P < 0.05$.

RESULTS

L-ornithine-L-aspartate effectively prevented the ethanol induced body and testes weight reduction (Table 1), however changes in testicular weight well correlated with body weight. Ethanol treated group demonstrated significantly high extent of lipid peroxidation in testes and decrease in testicular ascorbic acid and reduced glutathione (Table 2). The activities of SOD, catalase, GSH-Red and Se-GSH-Px were found to be significantly decreased in ethanol treated groups. Testicular activity of GSH-ST was significantly enhanced by ethanol

Table 1: Effect of exogenous L-ornithine-L-aspartate on mean body weight ($\pm$ SD) and mean weight ($\pm$ SD) of the testis.

Parameters	% increase in body weight	Weight of testis	
		In grams	In grams per body weight
Group I	42	0.781 $\pm$ 0.073	0.512 $\pm$ 0.003
Group II	17	0.623 $\pm$ 0.063*	0.508 $\pm$ 0.004
Group III	22.9	0.795 $\pm$ 0.063 ^{@, #}	0.506 $\pm$ 0.003
Group IV	21.38	0.782 $\pm$ 0.069 ^{@, #}	0.506 $\pm$ 0.003
Group V	26	0.750 $\pm$ 0.068*	0.507 $\pm$ 0.003

* $P < 0.05$ compared with control group. [@] $P < 0.05$ compared with ethanol treated group. [#] $P < 0.05$ compared with abstained group.

treatment (Table 3). In addition ethanol significantly reduced activities of testicular 3 β -HSD and 17 β -HSD (Table 4).

Drug exhibited an ability to counteract ethanol induced oxidative challenge as it effectively reduced testicular TBARS and increased tissue, ascorbic acid, GSH (Table 2), and activities of SOD, catalase, GSH-Red and Se-GSH-Px

Table 2- Effect of exogenous L-Ornithine-L-Aspartate on tissue levels of protein, ascorbic acid, thiobarbituric acid reactive substances and reduced glutathione

	Protein ¹	Ascorbic acid ²	TBARS ³	GSH ⁴
Group I	21.89 $\pm$ 0.83	1.86 $\pm$ 0.159	15.27 $\pm$ 0.30	2.14 $\pm$ 0.04
Group II	19.27 $\pm$ 0.30*	1.69 $\pm$ 0.058*	20.82 $\pm$ 0.13*	1.55 $\pm$ 0.04*
Group III	19.97 $\pm$ 0.20*, ^{@, #}	1.71 $\pm$ 0.031	18.85 $\pm$ 0.30*, ^{@, #}	1.75 $\pm$ 0.06*, ^{@, #}
Group IV	19.89 $\pm$ 0.31*, ^{@, #}	1.70 $\pm$ 0.013	19.05 $\pm$ 0.11*, ^{@, #}	1.74 $\pm$ 0.08*, ^{@, #}
Group V	19.31 $\pm$ 0.11*	1.70 $\pm$ 0.037	20.27 $\pm$ 0.56*	1.56 $\pm$ 0.06*

* $P < 0.05$ compared with control group. [@] $P < 0.05$ compared with ethanol treated group. [#] $P < 0.05$ compared with abstained group.

¹ mg/100mg tissue, ² mg/gm tissue, ³ nmol/mg tissue/min, ⁴ μ g/mg tissue

(Table 3). Drug had an opposite effect on the activity of GSH-ST. The effect of drug on 3 β -HSD and 17 β -HSD was not promising (Table 4).

DISCUSSION

Similar to our previous study chronic ethanol intoxication resulted in body and kidney weight reduction, oxidative stress and inhibited steroidogenic enzymes, 17 β and 3 β HSD. Treatment with L-ornithine-L-aspartate exhibited an ability to counteract the ethanol induced changes in the testes in

Table 3: Effect of exogenous L-ornithine-L-aspartate on tissue activities of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and glutathione S transferase

	SOD ¹	Catalase ²	GSH-Red ³	Se-GSH-Px ⁴	GSH-ST ⁴
Group I	21.18 ± 0.93	2.03 ± 0.10	1.61 ± 0.04	0.171 ± 0.02	11.53 ± 0.64
Group II	15.39 ± 0.65*	1.60 ± 0.54*	1.34 ± 0.04*	0.125 ± 0.01*	13.69 ± 0.79*
Group III	15.71 ± 0.35*,@	1.70 ± 0.05*,@,#	1.37 ± 0.02*,@,#	0.141 ± 0.01*,@	12.67 ± 0.39*,@
Group IV	15.63 ± 0.3*	1.69 ± 0.03*,@,#	1.35 ± 0.02*	0.139 ± 0.01*,@	12.88 ± 0.31*
Group V	15.73 ± 0.16*	1.64 ± 0.03*	1.32 ± 0.02*	0.139 ± 0.01*	12.89 ± 0.33*

Mean ± SD. * P<0.05 compared with control group. @ P<0.05 compared with ethanol treated group. # P<0.05 compared with abstained group. ¹ μmol pyrogallol auto oxidized /mg protein/min, ² nmol H₂O₂ decomposed/mg protein/min, ³ nmol NADPH oxidized/mg protein/min, ⁴ μmol CDNB conjugate formed/mg protein/min

preventive and therapeutic models in varying degrees. Reduction in testicular TBARS level in drug treated animals supports its antioxidant role. Raised levels of enzymatic and non enzymatic antioxidants in the drug treated animals elicit protective response.

Table 4: Effect of exogenous L-ornithine-L-aspartate on tissue activities of D⁵, 3β- and 17β- hydroxy steroid dehydrogenase (Mean ± SD)

	17 β- HSD ¹	3 β- HSD ¹
Group I	0.0166 ± 0.001	0.0198 ± 0.002
Group II	0.0131 ± 0.001*	0.0155 ± 0.001*
Group III	0.0131 ± 0.001*	0.0158 ± 0.001*
Group IV	0.0131 ± 0.001*	0.0156 ± 0.001*
Group V	0.0130 ± 0.001*	0.0156 ± 0.001*

n=6; *P<0.05 compared with control group;

¹ absorbance/mg protein/min

Explanations against the possible mechanism underlying the protective properties of drugs include the prevention of GSH depletion (18) and destruction of free radicals (19). Although the controlled generation of reactive oxygen species (ROS) serves as a second messenger system in many different cell types, its continuous production is detrimental to the surrounding tissue (20). It is reported that excessive ROS production beyond critical levels overwhelm antioxidant defense strategies of spermatozoa in seminal plasma results in increased oxidative stress. ROS are regularly formed during the normal respiration. However, the production is kept at physiologically low levels by intracellular free radical scavengers (21). GSH is a major thiol in living organisms, which plays a central role in coordinating the body's antioxidant defense processes. The tissue GSH concentration reflects its potential for detoxification, preserving the proper cellular redox balance and it's a role as a cellular protectant (22). GSH-Red is concerned with the maintenance of cellular level of GSH by

effecting the fast reduction of oxidized glutathione (GSSG) (23). GSH-Px plays a major role in peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes, spermatogenesis, sperm morphology and sperm motility (24). The increased tissue GSH-ST activity on ethanol treatment might be an adaptive mechanism as it plays an essential role in eliminating toxic compounds by conjugation (25).

However, L-ornithine-L-aspartate supplementation showed a minimal effect on reversing the inhibitory effect of ethanol on steroidogenesis. The antioxidant effect of L-ornithine-L-aspartate against oxidative stress induced by ethanol could have contributed some effect on these enzymes. This possibility is supported by the fact that L-ornithine-L-aspartate reversed the extent of lipid peroxidation and restored the testicular scavenger enzymes against free radicals. Low level of testicular free radicals in drug-treated rats was further supported by the recovery of testicular scavenger enzymes against free radicals (26, 27) Gain in the testicular weights in drug-treated rats also supports the low TBRAS and increased activity of steroidogenic enzymes. (28)

From the results it may be concluded that L-ornithine-L-aspartate has a protective effect on ethanol-induced testicular oxidative stress. The treatment showed only minimal effect in restoring steroidogenesis.

REFERENCES

1. Maneesh M, Jayalekshmi H, Dutta S, Chakrabarti A, Vasudevan DM. Effect of chronic ethanol administration on testicular antioxidant system and steroidogenic enzyme activity in rats. *Ind J Exp Bio* 2005; 43: 445-9.
2. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed. Cambridge Univ. Press, Cambridge; 1992.

3. Maneesh M, Jayalekshmi H, Dutta S, Chakrabarti A, Vasudevan DM. Experimental therapeutical intervention with ascorbic acid in ethanol induced testicular injuries in rats. *Ind J Exp Biol* 2005; 43: 172-6.
4. Stauch S, Kircheis G, Adler G. Oral L-ornithine-L-aspartate therapy of chronic hepatic encephalopathy: results of a placebo-controlled double-blind study. *J Hepatol* 1998; 28(5): 856-64.
5. Van Thiel DH, Lester R, Vaitukaitis J. Evidence for a defect in pituitary secretion of luteinizing hormone in chronic alcoholic men. *J Clin Endocrinol Metab* 1978; 47(3): 499-507.
6. Sidney K. Blood alcohols levels and fatal traffic accident. *Bol Assoc Med de P R* 1969; 61: 244-52.
7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.
8. Sinnhuber RO, Yu TC, Yu TC. Characterization of the red pigment formed in the thiobarbituric acid determination of oxidative capacity. *Food Res* 1958; 23: 626-30.
9. Roe JH, Kuther CA. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenyl hydrazine derivative of dehydroascorbic acid. *J Biol Chem* 1943; 147: 399-407.
10. Ellman G. The sulphhydryl groups. *Arch Biochem Biophys* 1959; 32: 70-77.
11. Mahley AC, Chance B. In: *Methods in Biochemical Analysis*. Ed. Glick, D (Wiley Inter Science, New York) 1954; pp 357
12. Marklund S, Marklund G. Involvement of superoxide radical in the autoxidation pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1974; 7: 469-74.
13. Paliga DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 1158-9.
14. Goldberg MD, Spooner JR. In: *Methods of Enzymatic Analysis* Ed. Bergmayer Vol.III, 3rd edn. (Academic press, Inc. Florida) 1983; 258-65.
15. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase, the first enzyme step in mercapturic acid formation. *J Biol Chem* 1974; 249: 7130-39.
16. Talalay P. Hydroxysteroid dehydrogenase, *Methods in Enzymology*, edited by S P Colowick and N O Kaplan (Academic Press: New York) 1962; 512-6.
17. Jarbak J. Soluble 17 β -hydroxy steroid dehydrogenase of human placenta, *Methods in Enzymology*. 1969; 15: 746-52.
18. Campos R, Garrido A, Guerra R, Valenzuela A. Silybin dihemisuccinate protects against glutathione depletion and lipid peroxidation induced by acetaminophen on rat liver. *Planta Med* 1989; 55: 417-9.
19. Valenzuela A, Lagos C, Schimdt K, Videlp LA Silymarin protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat. *Biochem Pharmacol* 1985; 3: 2209-12.
20. El-Seweidy MM, Hashem RM, Abo-El-matty DM, Mohamed RH. Frequent inadequate supply of micronutrients in fast food induces oxidative stress and inflammation in testicular tissues of weanling rats. *J Pharm Pharmacol* 2008; 60(9):1237-42.
21. Gumieniczek A, Hopka H, Zabek A. Protective effects of a PPARgamma agonist pioglitazone on anti-oxidative system in testis of diabetic rabbits. *Pharmazie* 2008; 63(5): 377-8.
22. Shalini S, Bansal MP. Co-operative effect of glutathione depletion and selenium induced oxidative stress on API and NFkB expression in testicular cells in vitro: insights to regulation of spermatogenesis. *Biol Res* 2007; 40(3): 307-17.
23. Sahoo DK, Roy A, Chattopadhyay S, Chainy GB. Effect of T3 treatment on glutathione redox pool and its metabolizing enzymes in mitochondrial and post-mitochondrial fractions of adult rat testes. *Ind J Exp Biol* 2007; 45(4): 338-46.
24. Zhang T, Chabory E, Britan A, Grignard E, Pitiot O, Saez F, et al. GP_x5, the selenium-independent glutathione peroxidase-encoding single copy gene is differentially expressed in mouse epididymis. *Reprod Fertil Dev* 2008; 20(5): 615-25.
25. Campbell A, Holstege D, Swezey R, Medina-Cleghorn D. Detoxification of molinate sulfoxide: comparison of spontaneous and enzymatic glutathione conjugation using human and rat liver cytosol. *J Toxicol Environ Health* 2008; 71(19): 1338-47.
26. Debnath D, Mandal TK. Study of quinalphos (an environmental oestrogenic insecticide) formulation (Ekalux 25 E.C) induced damage of the testicular tissues and antioxidant defense systems in Sprague-Dawley albino rats. *J Applied Toxicol* 2000; 20: 197-204.
27. Chainy GBN, Samantaray S, Samanta L. Testosterone-induced changes in testicular antioxidant system. *Andrologia* 1997; 29: 343-9.
28. Trasler JM, Hales BF, Robaire B. Chronic low dose cyclophosphamide treatment of adult male rats: effect on fertility, pregnancy outcome and progeny. *Biol Reprod* 1986; 34: 275-83.